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Increased conditioned immobility and weight loss in rats following mechanical impacts to the skull that do not produce loss of consciousness

Research Article

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Abstract: Rats either received a single vertical impact (15 km/h) of mechanical energy to their right dorsal skulls over the parietal region or served as handled controls. About 50% of the rats appeared normal after the impact. Thirty days later there were conspicuous areas containing neurons with shrunken and darkly stained somas within the cortices beneath the impact site and within the amygdala and entorhinal cortices. These neurons, occupying an average total area that ranged from 0.50 mm^2 to 5 mm^2, were evident even in rats that showed no stunning following the impact. These neurons were not seen in control rats. Subsequent decreases in body weight for rats that received the impact (even with no obvious stunning) were attenuated by oral access to 10% glucose but not by treatment with acetaminophen or ketamine. The rats that sustained the impact also displayed increased immobility within settings with which an aversive stimulus had been associated. Post-impact injection with ketamine did not normalize this behaviour. These results show that quantitative changes in some neuronal soma remain weeks after a single impact of mechanical energy that is not associated with immediate changes in behaviour. Concomitant with these neuronal alterations was increased emotional responsiveness to contexts associated with a single aversive episode and transient decreases in body weights.

Keywords: *Mechanical impact • Conditioned fear • Weight loss • Darkly-stained neurons • Limbic system • Parietal cortices*

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1. Introduction

Changes in behaviour following mild head injuries from a single impact of mechanical energy have been reported for wild and domestic animals, including human beings. Following a single Cranial Mechanical Impact (CMI) many human beings display debilitating affective (emotional) disorders such as anxiety and depression that may not be evident during casual observation. The insidious effects of affective disorders [1] upon post-CMI physical and intellectual capacities influence the individual's subsequent socialization and integration within its peer groups.

Robinson and Jorge [2] postulated that affective disorders are due to dynamic changes in the brain, mediated by neurotransmitters and neurohormones as a consequence of the impact of mechanical energy. Generally, there are disruptions in epinephrine, norepinephrine, dopamine [3], acetylcholine [4], endogenous opiates, glutamate, and corticotrophin releasing factor [5]. Diminished metabolism of neurons, depending upon location, also plays a prominent role in post-CMI depressive disorders [6]. Symptoms labelled as depression are usually more common among individuals with right hemispheric disruptions.

We have found that a single impact of mechanical energy produced disruptions in spatial learning and memory (but not in routine or "procedural" behaviours such as ambulation) even though there was no evidence of "stunning" or loss of consciousness. The impact was delivered from a 200 g weight dropped from a height

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0.9 m (impact velocity of 4.2 m/s or 15 km/h) upon the top of the right side of a rat's caudal skull. The calculated force of the impact was about 2 N. The amplitude of the pressure wave was estimated to have varied between 3 kPa and 20 kPa with a transit time through the brain of about 2 ms to 3 ms. These pressures are similar to those commonly estimated during predator-prey interactions, where the latter escapes despite significant impact to the head delivered from the predator.

A force of about 2 N applied along the 2 cm thickness of the rat cerebrum would generate an energy of 4 x 10^{-2} J. Assuming a cell width of 10 μm, the energy equivalent would be about two pJ per soma volume. For comparison, the amount of energy produced by glucose metabolism within this volume of a soma (assuming a typical human brain metabolic rate of about 35 (SD=4) micromole of glucose per 100 g of brain tissue per min) is also in a similar order of magnitude of picoJoules. Even theoretical estimates predict that cytoplasmic machinery of some neurons might be vulnerable to the effects of this sudden energy burst. We hypothesized that the passage within 2 ms to 3 ms of this energy through the whole soma would disrupt but not destroy the function of organelles within the cytoplasm of the most vulnerable neurons.

The mechanical impact results in conspicuous morphological changes (even at 40 X). The changes occur in clusters of neurons within the cerebral cortices immediately below the impact site and in the "countercoup" areas within the piriform lobes, amygdala, and entorhinal cortices. These neurons, which are still present for more than 60 days after the impact, are darkly-stained with obviously shrunken somas. They are distributed between neurons with normal somas. Within the limits of conventional histological stains and light microscopy, the anomalous somas appear to be an intermediate stage between apoptosis and necrosis. Their presence, with minimal evidence of decline, two months after the trauma indicates that cell surface proteins are still integrated and phagocytosis has not been initiated. We suspect this somatic morphology reflects a state of dormancy.

Since the trauma in our experiments was localized to the dorsal right part of the rats' skulls, we hypothesized the emergence of the rat-equivalent of human affective disorders which can affect "cognitive" performances. For example, fear sensitization or anxiety can occur as a consequence of recurrent temporal lobe seizures [7,8]. The numbers of neurons initiating these overt seizures may be minute compared to the total population of neurons. The resulting sensory-limbic hyperconnection results in heightened emotional reactivity to neutral stimuli [9]. Previous studies have demonstrated that rats display marked fear sensitization after kindling of the amygdala [8].

The conditioned fear paradigm is an example of classical conditioning in which a rat learns to associate a stimulus (S) with an unconditioned stimulus (UCS) (e.g., an electric shock). The conditioned response (CR), like the UCR, is protracted immobility that is usually labelled "freezing" behaviour [9]. Before it is associated with the UCS, the stimulus (or context) does not elicit any freezing behaviour. The freezing behaviour is in response to the conditioned context (CS) and is considered an adaptive mechanism that allows a rat to avoid detection by predators allowing it to escape more easily [10].

In the present study, CMI and control animals were injected with ketamine immediately after the mechanical impact and the conditioned fear paradigm was tested. A single injection of ketamine (100 mg/kg), a non-specific NMDA antagonist, has been found to facilitate recovery following the experimental induction of epileptic seizures and to improve learning of conditioned taste aversion and fear conditioned analgesia [11]. We thus injected this pharmacological agent in an attempt to improve behavioural adaptability and facilitate the animals' recovery from the CMI.

In a previous study we [12] measured a loss (about 5%) of body weight in CMI female rats during the first week after the mechanical impact even though there was no evidence of post-concussional stunning. In humans, these weight losses may go unreported or may be attributed to complications from medication, surgery, or hospital food. Weight loss has been attributed as well to diminished appetite, connected with depression mediated by disruptions of neurotransmitters and neurohormones or altered nociception. However, the actual cause of the weight loss is unclear.

In the present study we measured the food consumption of CMI and control animals. We measured this before and after impact, and after they had been treated with or without ketamine, acetaminophen in the water supply, or 10% glucose in food mush *ad libitum*. Acetaminophen and glucose have been shown previously to increase the rate of the rats' return to baseline weights after seizures were induced by a single systemic injection of lithium and pilocarpine [13]. Persinger [14] found that rats with histories of epileptic seizures and self-inflicted injuries were capable of discerning 'pain' and of self managing this inferred state with acetominophen when they were given a choice between this analgesic and tap water. Therefore, if the animals were "experiencing" increased nociception after a CMI one would predict they would select more antinociceptive solution in a two bottle (water vs. analgesic) choice paradigm.

2. Experimental Procedures

2.1. Subjects

A total of 52, 18 to 22 month old female Wistar rats served as subjects. Rats were obtained from Charles River, Quebec at approximately 60 days of age and were housed in threes before the experiments in wire or plastic shoebox cages. The animals had access to Purina rat food and water *ad libitum*. The ambient temperature in the colony was maintained at 20°C ± 1°C. The light: dark cycle was 12:12 with a photophase onset at 07:30 h. Animals were maintained with strict adherence to Canadian Council for Animal Care (CCAC) guidelines and with the approval of the local Animal Care Committee.

2.2. General Procedure

Animals were randomly divided into two experimental groups: control and Cranial Mechanical Impact (CMI). The CMI group was subjected to a procedure that has been described elsewhere [12]. Briefly, the rats were restrained by hand. Their heads were rested on a Formica-surfaced table. A weight of 200 g was dropped from a height of 0.9 m through a cardboard tube onto the right dorsal surface of their heads, approximately over the parietal region. Immediately after the CMI and when the animals had fully recovered (maximum time 2–3 min) as judged by their normal behavioural repertoire (ambulation, sniffing, and normal righting reflexes), they were returned to their home cages. Some animals that received the mechanical impact were stunned mildly and exhibited decreased mobility for 30 seconds (ranked 2) or less, while others showed diminished reflexes and immobility for 2 to 3 min (ranked 3). A third category (about 50%) did not display any evidence of stunning (ranked 1). Control rats that were held in the same manner but received no impact were ranked as 0.

2.3. Specific Procedure

In Experiment 1, the body weights and food consumptions of 7 rats were measured once per day for three days. Four rats sustained the mechanical impact to the skull while the other three served as controls. Body weights and food consumptions were then measured for another seven days.

In Experiment 2, five rats that had received the mechanical impact and four controls were trained and tested in a contextual, conditioning fear paradigm. The fear conditioning consisted of three scrambled footshocks (0.5 mA, 2 s, interstimulus interval 60 s) delivered 3 min after habituation to the chamber. This occurred on days 0 and 18. Animals were then observed (no shock delivered) in the experimental chamber every two days after the training session for four weeks. We observed and recorded movement (0) or freezing (1) every 8 s for 8 min (*i.e.* total of 60 observations per session). After each rat was tested, olfactory cues were masked by wiping the chamber with 0.4% acetic acid.

In Experiment 3, 16 rats that received the mechanical impact (n=8) or control (n=8) treatments were injected subcutaneously (hind flank) and immediately with either 100 mg/kg of ketamine (n=4) or isotonic saline (n=4). Food consumption and body weights were measured for 14 days. They were then trained and the fear conditioning paradigm was tested.

In Experiment 4, body weights were measured daily for two days. Five rats received the mechanical impact and four served as controls. Two separate 100 ml graduated bottles containing Tylenol (1 mg/ml in tap water) or tap water were made available *ad libitum* for each rat for the subsequent week. Daily fluid consumptions were measured and body weights were obtained every second day for one week.

In Experiment 5, after two days of body weight measurements, another group of rats sustained the mechanical impact (n=6) or served as controls (n=5). Food mush containing 10% glucose was made available *ad libitum* in small containers within the home cages (as well as dry food in the bins and regular water) for one week. Body weights were measured every second day.

Thirty days after the impact, rats were randomly selected from each of the first four experiments on the basis of their post-impact behaviours: (1) no stunning (n=4), (2) mild stunning (n=3) and (3) maximum stunning (n=3). Rats were removed from their cages and decapitated by guillotine within 10 s**.** Their brains were removed within 4 min, fixed immediately in ethanol-formalin acetic acid, and processed for paraffin embedding. Coronal sections (10 micrometers) were obtained by microtome between the posterior and anterior commissure. Sections were then stained with toluidine blue O. Ten sections, equally spaced between the two commissures, were assessed by light microscopy for the areas containing anomalous neurons. Each area was 0.026 mm^2 which was one square in a 6 x 6 grid (36 squares) at 100 x.

Typically, in the affected area there were 3 to 4 neurons with conspicuous somas. Their volumes were reduced by approximately half the normal value and they contained a darkly stained Nissl substance. They were distributed within a comparable number of normal neurons per square. The sum of the total numbers of squares containing aberrant neuronal morphology was calculated for the left and right sides of the brain separately. Since more than 99% of these cells were apparent within the cerebral cortices and the ventral lobes (the temporal,

entorhinal, retrosplenial and pyriform cortices and the amygdala and endopiriform nuclei) the data were divided into the resulting four sectors: upper and lower left and right quadrants.

2.4. Statistical Analyses

Net differences and relative differences in body weight were calculated for the second and fourth days after the treatment by subtracting these values from the baseline body weight, dividing the net differences by the baseline weight and multiplying by 100%. There were no statistically significant differences between the levels of stunning for baseline body weights (grand mean=373 g; SD=60 g).

To discern the quantitative significance of the intracerebral distribution of anomalous areas of neurons, three-way analyses of variance were completed for the rats that sustained the injury. This three way ANOVA was conducted with two within subject levels (left vs. right side of the brain; dorsal cortices vs. lower regions) and one between subjects level (stun levels 0, 1 and 2). We examined four randomly sampled control rats to verify that control rats did not have the anomalous neurons.

Intercorrelation and exploratory factor analyses were conducted between the total areas containing anomalous cells in the various areas and between the four (left vs. right; dorsal vs. ventral) areas of the brain. To minimize spurious results, the criterion for acceptance of loading coefficients (r values) as statistically significant was raised from 0.40 to 0.70. To detect any relationship between weight loss and size of the intracerebral areas containing anomalous cells within various regions, both nonparametric (Spearman rho) and parametric (Pearson) correlations were completed. Eta-squared values were included to discern the amount of variance in the dependent variables that could be explained by the CMI treatments and level of stunning. All analyses involved SPSS on a VAX 4000 computer.

3. Results

3.1. Experiment 1

A two-way analysis of variance (ANOVA), with one level repeated (relative differences in body weights over days) and one level not repeated (control vs CMI), demonstrated statistically significant differences between conditions [$F(1,5)=10.74$, $p<0.05$, $\eta^2=68\%$] and days [$F(4,20)=5.49$, $p<0.05$, $\eta^2=52\%$]. There was no interaction ($p>0.05$) between conditions and days. The CMI animals (M=-5.98%, SEM=1.03%) lost more weight overall than did the controls (M=-1.71%, SEM=0.55%) during the week subsequent to the impact.

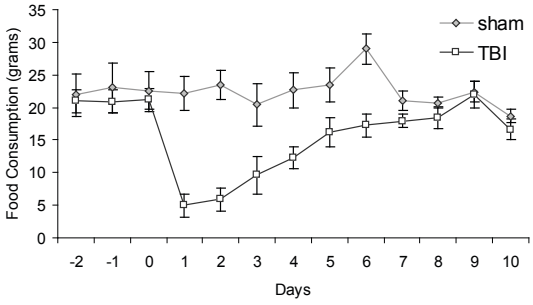

Figure 1. Food consumption of rats that sustained the CMI (cranial mechanical impact) or TBI (from an impact of 15 km/h) and control animals over days (impact occurred on day 0). Vertical bars correspond to standard error of the mean (SEM).

An ANOVA with one level repeated (food consumption over days) and one level unrepeated (conditions) showed a statistically significant interaction [$F(12,60)=6.92$, $p<0.05$, $\eta^2=58\%$] between conditions and days. There was also a statistically significant difference [$F(12,60)=7.21$, $p<0.05$, $\eta^2=59\%$] between days. *Post hoc* analyses indicated that the CMI animals' food consumption dropped significantly per day after the trauma, relative to the control animals (Figure 1). Overall, the CMI animals consumed less food per day than did the control animals (M=15.72 g, SEM=0.84 g vs. M=22.34 g, SEM=2.08 g).

3.2. Experiment 2

The data for avoiding a context associated with a traditionally "painful" stimulus, after transforming the raw data to percentages, were analyzed by a two-way ANOVA with one within subject level (days) and one between subject level (conditions). It yielded a main effect for condition (CHI vs control) that approached statistical significance ($p=0.06$) with a two-tailed test. However, it was statistically significant ($p<0.03$) if our *a priori* hypothesis of enhanced conditioning was considered. There was a statistically significant interaction [$F(14, 98)=13.17$, $p<0.05$, $\eta^2=20\%$] between conditions and days. *Post hoc* analysis indicated that CMI animals exhibited more immobility ("freezing") than did control animals over the testing period (Figure 2), particularly during days 14 to 18 when control rats were beginning to habituate to the context.

3.3. Experiment 3

A three-way ANOVA with one level repeated (relative body weight over days) and two levels not repeated (condition: CMI vs control; drugs: ketamine vs saline) did not show statistically significant differences ($p>0.05$) between CMI or drug conditions. No significant

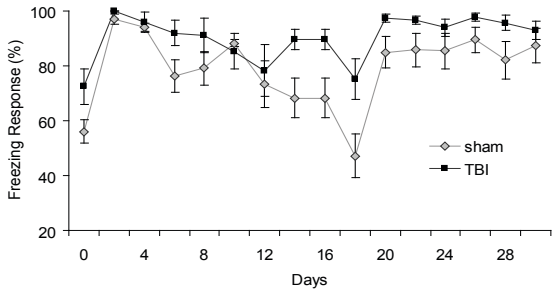

Figure 2. Freezing behaviour (%) of both CMI (TBI) and sham (control) animals over 30 days. Vertical bars denote SEM. Animals were fear conditioned (with shock) on days 0 and 18. The other days refer to testing in the context (CS) but without the shock.

interactions (p>0.05) were found between conditions and drugs, drugs and days, or conditions, drugs and days. However, there was a statistically significant two-way interaction [$F(7,77)=3.54$, $p<0.05$, $\eta^2=24\%$] between conditions and days. *Post hoc* analyses revealed that, irrespective of ketamine or saline, the CMI animals lost significantly more body weight relative to the control animals subsequent to the impact.

An ANOVA with one level repeated (food consumption over days) and two levels not repeated (conditions and drugs) showed a statistically significant main effect [$F(1,11)=5.06$, $p<0.05$, $\eta^2=32\%$] for conditions, but not ($p>.05$) for drugs. No interaction ($p>0.05$) for conditions by drugs was apparent. There was a statistically significant difference [$F(6,66)=11.02$, $p<0.05$, $\eta^2=50\%$] between days. The CMI animals exhibited decreased food consumption following the trauma relative to control animals. On average the food consumption of the control animals (M=43.66 g, SEM=1.44 g) was more than the CMI animals (M=36.95 g SEM=2.22 g).

After the post-CMI data were transformed into ratios (by dividing the raw scores by the baseline to minimize individual variations), a three-way ANOVA with one level repeated (days of fear conditioning) and two levels not repeated (CMI vs sham; ketamine vs saline) revealed significant differences between conditions [$F(1,11)=4.91$, $p<0.05$, $\eta^2=31\%$], and days [$F(7,77)=7.63$, $p<0.05$, $\eta^2=41\%$]. A two-way interaction [$F(7,77)=2.42$, $p<0.05$, $\eta^2=18\%$] between CMI conditions and days was also found. No other interactions were significant. *Post hoc* analyses found that overall CMI rats (M=2.38, SEM=0.18) were more immobile ("froze" more) than control animals (M=1.78, SEM=0.18), regardless of drug condition.

3.4. Experiment 4

A two-way ANOVA with one level repeated (body weight over days) and one between subject level (for net relative body weight changes) showed significant differences between conditions [$F(1,7)=6.68$, $p<0.05$, $\eta^2=49\%$] and days [$F(3,21)=3.40$, $p<0.05$, $\eta^2=33\%$]. There was a two-way interaction [$F(3,21)=3.23$, $p<0.05$, $\eta^2=32\%$] between conditions and days. *Post hoc* analyses revealed that the CMI animals lost significantly more body weight relative to the control animals subsequent to the trauma from days six to eight. On average, the CMI animals (M=-3.22%, SEM=0.63%) lost more weight than did control animals (M=-1.32%, SEM=0.15%). Fluid consumption for either the Tylenol-water or tap water, or the ratio of Tylenol-water to tap water did not show any significant differences ($p>0.05$) between groups (both control and CMI), even when the data were transformed to relative measures to accommodate individual variability.

3.5. Experiment 5

Preliminary analysis did not find any significant differences ($p>0.05$) in body weights between CMI or control rats that had been given food mush containing 10% glucose. A two-way ANOVA with one level repeated (body weight over days) and one level not repeated (conditions) did not reveal a main effect ($p>0.05$) for conditions or days. Furthermore, there was no two-way interaction ($p>0.05$) between conditions and days. Even when the data were transformed to accommodate individual differences, there were no statistically significant group differences.

3.6 Neurohistological Analyses

No anomalous neurons were observed within the coronal sections of the control rats. For rats that sustained the mechanical impact, the longitudinal extent of the areas containing anomalous cells within the cerebral cortices (below the impact site) was along the entire region sampled. This region extended between the occipital and caudal frontal cortices for the right hemisphere and was equivalent to Paxinos and Watson's [15] plates 19 to 54. The anomalous cells with the conspicuous shrunken and darkly-stained somas were found in areas as far laterally as the interface with the temporal cortices. The medial extent was more restricted and specific to the region immediately adjacent (within about 50 μm) to the longitudinal fissure within the parietal and frontal lobes (plates 19 through 36) in the left hemisphere. About 90% of these areas occurred in a coronal thickness corresponding to the maximum appearance of the subcortical structures of the ventromedial hypothalamus, medial habenulae, and amygdala (plates 33 through 34).

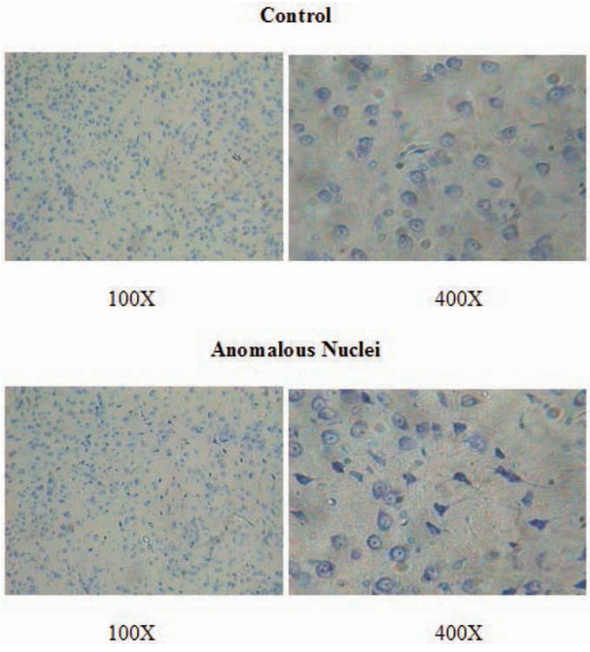

Figure 3. Examples of normal (above) nuclei from the left hemisphere and anomalous neuronal soma (below) from the right hemispheres at both low (100x) and high magnifications (400x) from a CMI rat's parietal cortices.

A three-way ANOVA with two within subject levels (left vs. right hemisphere; dorsal vs. ventral structures) and one between level measure (stun rating: 0, 1, 2) supported our hypothesis that rats exhibiting mild stunning (rating 1). These rats displayed the largest areas of anomalous cells [$F(2,7)=4.23$, $p<0.05$; one tailed test]. The means and standard errors of the mean for the total numbers of squares (each square=0.026 mm^2) containing anomalous neurons (Figure 3) per coronal section throughout the brain were 25.3 (2.0) for non stunning, 33.7 (5.2) for mild stunning, and 19.7 (4.6) for obvious stunning, respectively. These values corresponded to total cross-sectional areas of 0.66 mm^2, 0.88 mm^2, and 0.51 mm^2, respectively.

The range in the sum of areas containing neurons with shrunken soma in an average section ranged between 0.1 and 1.5 mm^2. However, at the level of the largest area of anomalous cells, which was within the parietal cortices (plates 33 through 36 in Paxinos and Watson), these values ranged between 1 and 4.8 mm^2. For comparison, the total cross-sectional area of a typical coronal section of a rat brain is about 120 mm^2. Only approximately half of the 0.025 mm^2 areas containing anomalous cells were immediately adjacent to other areas containing these cells. Percentages of neurons with anomalous somas within each punctuate area ranged between 30% and 50%.

Anomalous neurons with shrunken soma and dense atypical Nissl staining [12] were found primarily (in order of magnitude from highest to lowest) within the parietal, adjacent frontal, retrosplenial, and occipital dorsal cortices. Within the ventral regions the anomalous neurons were found in the amygdala, entorhinal cortices, piriform lobe, endopiriform area, and temporal cortices as delineated by Paxonis & Watson [15]. There were no anomalous neurons within the intermediate structures such as the thalamus or hippocampus.

The means and the standard deviations (in parentheses) for the sum of the areas containing anomalous neuronal soma in the four major regions were: upper left dorsal cortices 0.06 (0.04) mm^2, right dorsal cortices 0.19 (0.08) mm^2, left lower regions 0.15 (0.09 mm^2, and right lower regions 0.24 (0.13) mm^2. There were significantly larger areas of neuronal anomalies in the right (impacted) hemisphere compared to the left [$(F1,7)=95.59$, $p<0.001$] and more in the lower regions than in the upper regions [$F(1,7)=9.53$, $p<0.01$]. The interaction between rank of stunning (1, 2, and 3) and area of anomalous neurons between the left and right side of the brain approached statistical significance [$F(1,7)=3.95$, $p<0.10$]. The interaction was due to the larger areas of anomalous neurons in the right lower regions (entorhinal, piriform, amygdala) for the rats that displayed mildly stunned behavior (0.38 mm^2) compared to those who displayed no stunning (0.18 mm^2) or were transiently impaired (0.20 mm^2) as defined by left-sided paresis for about 100 s.

Exploratory factor analyses revealed two shared sources of variance (*i.e.*, factors). The first factor (eigen value 1.81; 47% of the variance explained) was loaded significantly (all rs >0.70) by the size of the area containing anomalous neurons in the left dorsal cortices (-0.70) and left ventral regions (0.95) of the brains. The second factor (eigen value=1.25, 31% of variance explained) was loaded by the areas of anomalous neurons within the right dorsal cortices (0.87) and right ventral region (-0.70).

The only statistically significant correlation (Spearman) between the two factor scores and the amount of weight loss following mechanical impact occurred with the right-sided factor. Spearman rhos of 0.56 ($p<0.05$) and 0.62 ($p<0.05$) were found between the net weight loss and relative (percentage) weight loss during the two days after the impact and increased areas of anomalous neurons within the limbic system. There were corresponding decreased areas of anomalous neurons within the dorsal cortices (immediately beneath the impact site) in the right hemisphere only.

Spearman correlations for the intercorrelations between the left and right hemisphere showed significant ($p<0.01$) values for the dorsal (primarily parietal and occipital) cortices (0.88), amygdala (0.84), and piriform

lobe (0.70). There were insignificant values for the left and right entorhinal cortices (0.04), endopiriform region (0.22), temporal cortices (0.08), and retrosplenial cortices (0.09). Partial correlation analyses indicated that when the variance associated with baseline body weights was removed, rats with more anomalous areas of neurons in the right dorsal cortices had exhibited *less* loss of body weight (partial r=-0.61, p<0.05). This effect was not evident for the left dorsal cortices (partial r=0.46) or for any of the ventral areas for either hemisphere. Spearman correlations with the relative changes in body weights verified this significant association for the right dorsal cortices.

In order to discern if specific areas within each of the regions along the neuroaxis were associated with the loss of weight, Spearman correlations were completed with the values for the sizes of the anomalous areas for each of the ten slides. The numbers of squares of anomalous neurons in the amygdala at the level where major fibers are sent to the ventromedial hypothalamus were correlated significantly (rho 0.78, 0.79) with the net weight loss for post-concussive days 2 and 4.

An exploratory multiple regression [limited to entry of only two variables (regions) to minimize artifactual inflation of the multiple-r] showed that the increased linear combination of numbers of squares of anomalous cells in the right temporal cortex and entorhinal cortices at the level where it receives input from the amygdala was associated with greater body weight loss (multiple r=0.93, F(2,7)=22.35, p<0.001]. The scattergram demonstrated a clear linear effect with no distortion from outliers.

4. Discussion

Mild and presumably ineffective impacts of mechanical energy to the skull occur frequently in all mammalian species due to the nature of prey-predator relationships and the inertial consequences of movement through space. Our results indicate that mechanical impacts producing no acute changes in animal behaviour could adversely affect fundamental longer term behaviours important for survival and adaptation to the social environment. In addition to losing body weight after an impact of mechanical energy to the skull, we found that rats remained more hypersensitive and fearful in contexts where aversive events occurred *only once*.

The enhancement of apprehensive responses was comparable to the effects of acute nicotine (0.25 mg/kg) in mice [16]. Our results are contrary to Hogg *et al.* [17] who found that more severe traumatic brain injury in the right parietal cortex resulted in deficits in conditioned emotional response, although others have found increased emotionality as well [18]. The differences in the results may be due to variations in the techniques by which the closed head injuries were induced, the durations of experiments, the lesion sites, and their sizes. The areas involved with the anomalous neurons in our study were miniscule in comparison to those associated with classical lesions. The occurrence of such bran damage in nature would more frequently be associated with failure to thrive unless there was sufficient support by species-specific social behaviours.

Anxiety (the learned anticipation of an aversive event) is an adaptive mechanism that allows an organism to avoid and to escape hostile conditions [8]. However, this “fear” becomes maladaptive if it interferes with the animal’s normal behaviours. CMI can be debilitating because it impedes the organism’s normal behaviour. Human animals that sustain CMIs and subsequently experience anxiety in addition to depression, post-traumatic stress disorder, and post-concussive syndrome are considered impaired. They are unable to cope with daily life because they are less able to regulate their anxiety and to cope with stressors. These individuals seem to be in a state of hyperarousal, nervousness, and tension; they startle and panic easily.

The amygdala in rodents has been implicated in the pathogenesis of emotionality. That the CMI rats displayed more “freezing responses” relative to controls within the contextual fear conditioning paradigm may be due to alterations of neuronal function of this structure. Increased emotionality in an open field test has been reported following amygdaloid kindling for both male and female rats [8]. In the present experiments, anomalous neuronal somas were discerned in the amygdala as well as the parietal, entorhinal, piriform, temporal, perirhinal cortices. Classic lesion studies have shown that several subcortical areas are involved in the expression of conditioned freezing behaviour within a context. These areas include the hippocampus, the basal nucleus (of Meynert) and the amygdala [19,20]. In the present study no overt lesions were observed in the hippocampus or the nucleus basalis.

Ketamine, an *N*-methyl-*D*-aspartate (NMDA) antagonist, when given immediately after the impact failed to reverse the emotionality associated with the CMI. Effectiveness may require ketamine to be given chronically. However, a single injection of ketamine administered in the same manner following the onset of limbic seizures in rats induced by a single injection of 3 mEq/kg of lithium chloride followed four hours later by 30 mg/kg of pilocarpine has been shown to be neuroprotective and to promote learning for procedures involving conditioned taste aversion, conditioned analgesia [11], the elevated plus maze, the open field,

and contextual fear testing [21]. These results are contrary to our findings. It may be that the processes involved with seizures are different than those induced by CMIs. Seizures are often associated with conspicuous neuronal loss which we did not observe in CMI rats.

In our previous study [12] exposure of rats to the fear conditioning paradigm before and after the trauma did not elicit any aberrant freezing response. It seems that in order for the numbers of freezing responses to be increased, animals must be exposed to the noxious context after the CMI. This behaviour may be analogous to human animals who sustain CMIs. They experience anxiety and panic attacks in contexts similar to the ones in which they received the head injury as well as in novel contexts.

Ketamine failed to prevent the weight loss subsequent to the CMI, suggesting that other processes were mediating these responses. In agreement with Jedrzejko and Persinger [13], we found that 10% glucose, but not acetaminophen, was effective in maintaining body weights after the CMI. Why ketamine and acetaminophen failed to assist the animals in maintaining and gaining their body weights may be due to a less than optimal dosage. However it may also be because the loss of body weight was not related to nociception secondary to the trauma. Some investigators have found that pain may not be an important factor regulating the general response to trauma [22].

The mechanism by which dietary glucose prevented the loss of body weight would be important to study further. It may have helped enhance the activity of the GABA (gamma aminobutyric acid) shunt. This source of GABA is in large part derived from the skeletal fragments of the glucose molecule. Glucose may alternatively have accommodated the increased cellular energy demands required to restore ionic membrane balance as a consequence of the period of mechanically induced neuronal excitation [23,24]. It is also possible the glucose may have increased the palatability of food for the CMI rats. If the latter interpretation is valid, then the post-impact weight loss would be similar to that associated with either lesions of the lateral hypothalamus or overstimulation of the ventromedial hypothalamus (VMH). In light of the strong correlation between weight loss and the total area of anomalous neurons within the amygdala at the level where major fibers directly influence the VMH, the latter explanation is more likely. Such a relationship would imply that the neurons with shrunken soma normally inhibited the VMH neurons. The sudden release from this tonic inhibition could have increased VMH activity, diminished food intake, and contributed to the weight loss.

We were careful to minimize distress to the rats while simulating the natural condition in which impacts of mechanical energy to the skull occurs. Neither wild animals nor human beings are typically anaesthetized during a mechanical impact. The rats did not display the physiological correlates of what is described as pain in human animals subsequent to the impact. There was an absence of preference for an oral anaesthetic known to be quickly selected in a dose-dependent manner by rats with varying areas of skin lesions that correlate with “levels of pain” [14].

In this and other studies, we have found that post-impact weight loss is a single strong predictor for the numbers of anomalous neurons beneath the impact site. Animals that have received the mechanical impact with or without being stunned all exhibited significant weight losses relative to control animals. We conclude that the critical indication of neuronal dysfunction may not be the absence or presence of consciousness following a closed head injury. Based on our findings, it may be prudent to reassess contemporary definitions for diagnosis of brain damage in human beings subsequent to even “mild” concussions. Glasgow Coma Scale scores above 12 may be more significant for long term adaptation than currently presumed.

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